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An emulsion blending approach for the preparation of Gelatin/Poly(butylene succinate-co-adipate) films

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ABSTRACT: Emulsion blending as a new method to combine a water soluble biopolymer, gelatin, with a synthetic biodegradable elastomer, poly(butylene succinate-*co*-adipate) (PBSA), was investigated. Blending by wet processing a hydrophilic biopolymer with a hydrophobic synthetic polymer aimed at evaluating the potential for improving the mechanical properties of the biopolymer without affecting its biodegradability. The effect of the variation of blend composition, and of the experimental procedure for the emulsification and the subsequent preparation of cast films from the resulting oil-in-water emulsions was analyzed. In particular, processing temperature, concentration of the precursor solutions (aqueous gelatin and PBSA in dichloromethane, respectively), blending method and post treatment conditions (T, P) affect the quality and stability of the aqueous gelatin emulsion containing PBSA in dichloromethane as the dispersed phase. Control of the aqueous phase viscosity is a key parameter for both the emulsion stability and the morphology of the final heterophasic cast films. In particular, viscosity must be sufficiently low to allow high shear emulsification, but high enough to prevent coalescence among the organic phase droplets. The process conditions optimized for a 80/20 blend were extended to the

preparation of blends with 5-30 wt% PBSA. It was found that evaporation of the organic phase must be nearly quantitative before casting to allow the formation of uniform films at any investigated composition of the immiscible polymer blend. In fact, when the films are produced by casting, the presence of residual organic solvent along with too high a viscosity of the aqueous gelatin phase promotes the formation of cavities opening up at the lower film surface as a result of the higher density of CH_2Cl_2. Such cavities, internally sheathed with PBSA microbeads precipitated upon evaporation of the organic phase, if smaller than 100 μm turned out to improve the flexibility of the films.

Introduction

Gelatin is a denatured protein obtained by partial hydrolysis of collagen.[1] It is usually extracted from animal collagen and is thus largely available either as a by-product or a waste from food and tannery industries.[1-2] The main use of purified gelatin is in food and pharmaceutical applications. However, gelatin from industrial waste effluents does not fit the safety requirements of these uses, therefore, there is interest to find other industrial applications. Gelatin may be a potentially interesting, eco-sustainable substitute of polymers of petrochemical origin, with obvious environmental and socio-economic advantages.[3-4] Its recovery and reuse would decrease both industrial waste production and oil consumption. In addition, gelatin is biodegradable and may be employed to formulate biodegradable plastics.[5]

Gelatin has excellent film forming properties.[1] However, its films are brittle and toughening is needed for most practical applications. This drawback can be tackled by blending with either low molecular weight species[6-11] or polymers[12-22] to provide plasticization and improve the ultimate mechanical properties. Among polymers, the most studied are poly(vinyl alcohol) (PVA)[12-13, 18, 20, 23-33] and chitosan[14, 19, 34-41] since they are both biodegradable and show good compatibility with gelatin, with which they can form hydrogen bonds. Blending is typically performed by wet processing in aqueous solution as both polymers are soluble in water like gelatin.

Blends of gelatin with hydrophobic thermoplastic polymers, such as poly(lactic acid) (PLA),[42-43] lactide copolymers,[44-45] polycaprolactone,[46-49] and polyolefins[16-17, 50-51] have also been reported. The blending process is less straightforward, as gelatin is soluble in a limited number of organic solvents such as dimethyl sulfoxide and a few fluorinated alcohols. Since the above synthetic polymers are generally soluble in halogenated solvents, blending is often performed in trifluoroethanol[47, 52-53] or hexafluoroisopropanol[54], which are also good solvents for gelatin. While melt processing of gelatin-based blends has been reported,[17, 33, 51, 55] wet processing would be highly preferred, as it is generally the case for the typically heat sensitive biopolymers.

Recently, a procedure involving the formation of an oil in water emulsion has been proposed to prepare biodegradable blends starting from an acidic aqueous solution of chitosan and a solution of PLA in chloroform. In fact the two polymers are soluble and insoluble in water, respectively,[56] similarly to any combination of gelatin with the commercially available biodegradable aliphatic polyesters. In the mentioned example, PVA was used as compatibiliser and emulsion stabiliser. In the case of a binary blend of gelatin with hydrophobic biodegradable polymers, on the other hand, the addition of a third component may result unnecessary since gelatin itself has been reported to have emulsion stabilising properties.[57-58]

Aim of this work was to explore and evaluate the emulsion blending method for preparing fully biodegradable blends of the hydrophilic gelatin with a hydrophobic polyester, and to study the thermal, morphological and mechanical properties of the resulting materials. As a representative hydrophobic polymer the aliphatic polyester poly(butylene succinate-*co*-adipate) (PBSA), commercially available as BionolleTM 3001, was selected. PBSA is characterized by fast biodegradability, low crystallinity (20-35 %), relatively high melting (T_m ~ 94 °C) and quite low glass transition temperature (T_g ~ -40 °C). Furthermore, it has low modulus and good elongation at break (600 %).[59-61] All the above features make PBSA a good candidate as the toughening phase in binary blends with gelatin. In fact, while improving its mechanical behaviour, it preserves the biodegradability and possibly improves the water resistance of gelatin films.

Materials and Methods

Materials

Type B gelatin (Gel) from bovine skin (bloom value 225, density 1.35 g/mL), 2,2,2-trifluoroethanol (TFE) and dichloromethane were purchased from Aldrich. Poly(butylene succinate-*co*-adipate) (PBSA) was a commercial product, tradename Bionolle 3001, obtained from Showa Denko Europe. Type I water (resistivity > 18 MΩ·cm) was obtained with a Millipore Direct Q3 UV apparatus.

Instruments and methods

Optical microscopy observations were performed with a Biolux NV instrument equipped with MikrOkular, PhotomizerSE software Motic Images Plus 2.0 for image collection and analysis.

IR spectra were recorded with a Jasco FT/IR-6200 spectrometer equipped with a Pike Technologies VeeMAX III Attenuated Total Reflectance (ATR) accessory mounting a ZnSe crystal. Background and sample spectra were collected by accumulating at least 32 scans. A reference PBSA film for analysis

was obtain by compression moulding at 180 °C between Teflon sheets for few minutes using a Carver mod. 3851CE plane plat press. All other films were obtained by solvent casting.

Tensile tests were performed on specimens cut from films according to ASTM D638 Type V standard. Analyses were carried out at room temperature, at a crosshead speed of 10 mm/min, with an Instron 4302 universal testing machine (Canton MA, USA) equipped with a 10 kN load cell and interfaced with a computer running the TestWorks 4.0 software (MTS Systems Corporation, Eden Prairie, MN, USA).

The morphology of the blends was studied with a JEOL JSM-5600LV (Tokyo, Japan) scanning electron microscopy (SEM); both film surfaces and cryofractured samples were analysed as such or after etching with dichloromethane at room temperature for 3 days; all samples were sputtered with gold.

Differential scanning calorimetry (DSC) analysis was performed with a Seiko SII ExstarDSC7020 calorimeter equipped with a liquid nitrogen-cooling unit. 5-8 mg of sample was analysed in aluminium pan under nitrogen atmosphere in a -70 – 150 °C temperature range. Both heating and cooling scans were carried out at 10 °C/min. The instrument was calibrated with indium (melting temperature and enthalpy: T_m=156,60 °C, ΔH_m=28,47 J/°C) and zinc (T_m=419,47 °C) as standards.

An Ultraturrax T®18 homogenizer operated at 22000 rpm was used to prepare oil-in-water emulsions.

Blending in TFE

Gelatin and PBSA solutions at 5 wt% concentration in TFE were prepared by stirring overnight at 40 °C and at room temperature, respectively. Gel/PBSA 80/20, 60/40, 40/60 and 20/80 blends were prepared by mixing the required amounts of each solution either under magnetic stirring or with an Ultraturrax homogenizer at 22000 rpm for 90 sec. The obtained mixtures were transferred into Petri dishes and allowed to dry at room temperature for several days. All samples were prepared at least in duplicate.

Optimization of emulsion blending condition

Gelatin and PBSA were solubilized by stirring in water at 40 °C overnight and in CH_2Cl_2 at room temperature, respectively. Solution concentrations for both polymers were 5 wt% and 10 wt %, respectively. Solution at lower concentration were prepared by dilution. Suitable amounts of each solution, depending on the given concentration, were mixed to prepare emulsions based on a gelatin/PBSA 80/20 dry blend composition. Mixing was performed under magnetic stirring or with an Ultraturrax homogenizer (see Table 1). A post-treatment step of mild stirring with magnetic rod, followed, for selected samples, by a sonication treatment in an Ultrasound bath at room temperature, was performed to stabilize the emulsions and remove trapped air bubbles.

The stability of the emulsions was visually evaluated from the occurrence of macroscopic phase separation over time in test tubes.

Preparation of films from emulsions

Depending on the desired blend composition, suitable amounts of 5 wt% gelatin solution in water (10 wt.% when specified) and 10 wt% (or lower when specified) solution of PBSA in CH_2Cl_2 were mixed with an Ultraturrax for 90 sec at room temperature (see Table 1). The obtained emulsions were further stirred at room temperature with magnetic rod (1 hour) and sonicated in an ultrasound bath (½ h) to remove air bubbles and to allow partial evaporation of the organic solvent.[56] The resulting stabilised emulsions were transferred into PTFE capsules and allowed to dry at room temperature to give free standing solid

Table 1: Experimental conditions adopted to prepare Gel/PBSA oil-in-water emulsions

Run[1]	Solution concentration[2]		Gelatin/ PBSA	Mixing			Post treatment			H_2O/CH_2Cl_2
	Gelatin (wt %)	PBSA (wt %)	wt%	Method[3]	Time (sec)	Temperature[4] (°C)	Method[3]	Time (min)	Temperature (°C)	vol. ratio
C_5	5	5	80/20	S	30	r.t.	S	30	r.t.	4
C_{10}	10	5	80/20	S	30	40	S	30	r.t.	2
C_5T_{60}	5	5	80/20	H	60	r.t.	-	-	-	4
$C_{10}T_{60}$	10	5	80/20	H	60	r.t.	-	-	-	2
$C_5T_{60}S_{60}$	5	5	80/20	H	60	r.t.	S	60	30	4
$C_5T_{90}S_{60}$	5	5	80/20	H	90	r.t.	S	60	r.t.	4
$C_5T_{120}S_{60}$	5	5	80/20	H	120	r.t.	S	60	r.t.	4
$C_3T_{120}S_{60}$	3	5	80/20	H	120	r.t.	S	60	r.t.	6
$C_5T_{90}S_{60}U_{30}$	5	5	80/20	H	90	25	S+U	60+30	25°C	4
$C_{5,10}T_{90}S_{60}U_{30}$	5	10	80/20	H	90	r.t.	S+U	60+30	r.t.	8
G100	5	-	100/0	H	90	r.t.	S+U	60+30	r.t.	n.a.
G100R	5	-	100/0	H	90	r.t.	S+U	60+30	r.t.	18
G95P5	5	10	95/5	H	90	r.t.	S+U	60+30	r.t.	38
G90P10	5	10	90/10	H	90	r.t.	S+U	60+30	r.t.	18
$G90P10_{4.4}$	5	4.4	90/10	H	90	r.t.	S+U	60+30	r.t.	8
$G90P10_{2.6}$	5	2.6	90/10	H	90	r.t.	S+U	60+30	r.t.	4.7
G90P10T	5	10	90/10	H	90	25	S+U	60+30	25	18
G80P20	5	10	80/20	H	90	r.t.	S+U	60+30	r.t.	8
G70P30	5	10	70/30	H	90	r.t.	S+U	60+30	r.t.	4.7
G70P30R[5]	5	10	70/30	H	90	r.t	S+U+V	60+30	r.t.	4.7

[1]Runs CnTmSpUq were performed to optimize emulsification conditions, while runs GxPy refer to emulsions actually used for film preparation; [2]Solutions of gelatin in de-ionized water and PBSA in dichloromethane, respectively; [3]Methods as follows: S = magnetic stirring, H = homogenization with Ultraturrax, U = ultrasonication, V= vacuum; [4]The temperature of gelatin solutions was 40°C before mixing; the temperature reported here refers to the condition during mixing; 5Emulsified blend treated in a rotary evaporator at ~18 mmHg and room temperature to remove the organic solvent before casting.

films. Faster evaporation of the organic solvent was induced for mixture G70P30R by using a rotary evaporator operated at 18 mmHg at room temperature during about 1 h, followed by film casting of the resulting aqueous suspension. In the case of sample G90P10T the mixture was thermostated at 25°C during both the homogenization and the post-treatment steps. All samples were prepared, at least, in duplicate to assess replicability.

Results

Blending in TFE

For a preliminary evaluation of the compatibility between gelatin and PBSA, 80/20, 60/40, 40/60 and 20/80 blends were prepared by using TFE as a common solvent for the two polymers. Solutions at 5.0 wt % concentration of each polymer were mixed under magnetic stirring, then binary blend films were obtained from the resulting mixtures by casting at room temperature onto Petri dishes. The polymer blends produced with the above procedure gave warped inhomogeneous films presenting both white-opaque and translucent areas (Figure 1). While pure gelatin gives smooth and transparent TFE-cast films, pure PBSA films were uniformly opaque, warped, spongy and brittle (Figure 1a), indicating incipient precipitation during solvent evaporation. Accordingly, white areas and warping degree increased with the amount of PBSA in the blends. ATR-FTIR analysis of the film surfaces clearly indicated the occurrence of phase separation across the film section, with gelatin as the almost exclusive component of the lower surface and PBSA as that of the main one on the upper surface (see Figure S1 in supplementary information). SEM analysis of cryofractured blends showed the presence of few scattered domains even in the transparent regions (see Figure S2 in supplementary information). Preparation of gelatin/PBSA blends by solvent evaporation in a rotary evaporator, instead of solvent casting and drying at atmospheric pressure, provided a more homogenous material on a macroscopic scale (Figure 1f).

The above-mentioned results indicated a strong incompatibility between gelatin and PBSA. Accordingly, DSC analyses showed no significant shifts of the glass transition temperature of PBSA phase when blended with gelatin (Table S1 in supplementary information).

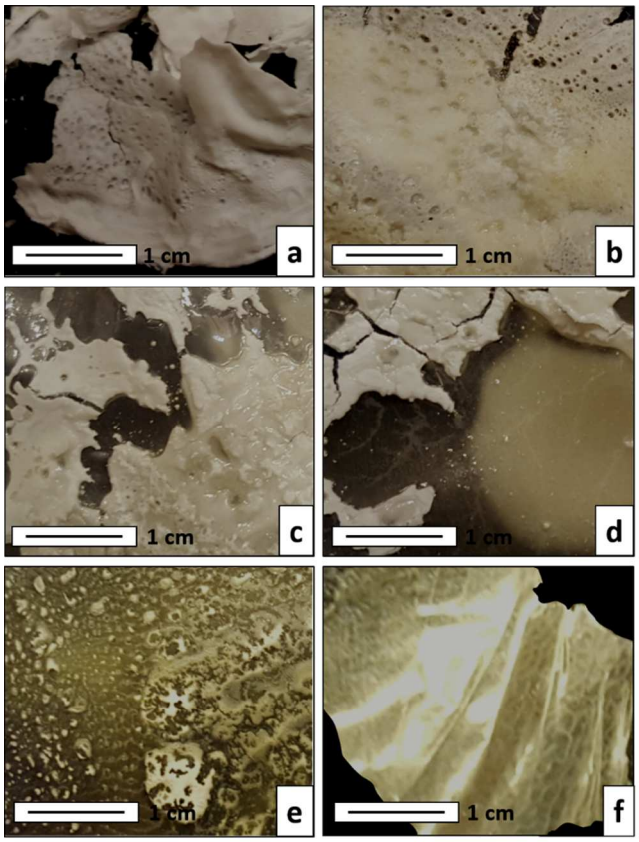

Figure 1. Optical images of gelatin/PBSA blends prepared from TFE solutions: (a) fragments of pure PBSA obtained by solvent casting (b) 20/80 blend film obtained by solvent casting; (c) 40/60 blend film obtained by solvent casting; (d) 60/40 blend film obtained by solvent casting; (e) 80/20 blend film obtained by solvent casting; (f) fragment of 80/20 blend obtained in rotary evaporator.

Blending in oil-in-water emulsion

In order to explore the alternative preparation methodology of blending gelatin (Gel) with PBSA in an aqueous medium, PBSA solutions in dichloromethane were mixed with aqueous gelatin pre-heated at 40 °C to ensure effective solubilisation. Preliminary experiments aimed at obtaining stable oil-in-water (o/w) emulsions were performed by blending at a constant Gel/PBSA 80/20 weight ratio (based on dry weight), while the polymer solution concentrations before emulsification, processing temperature during emulsification, and homogenization processing time were varied (see Table 1).

Emulsions C_5 and C_{10} were prepared by dropwise addition of the PBSA solution to the gelatin solutions maintained under simple stirring with a magnetic rod. Experiment C_5 was performed at room temperature (after solubilisation of gelatin with gentle heating) while in experiment C_{10} the temperature had to be raised to 40 °C, since at room temperature the 10 wt% aqueous gelatin phase would undergo a gelling transition during the mixing procedure. Homogeneous emulsions were obtained after 30 minutes of stirring in both C_5 and C_{10} experiments; however, the resulting emulsions were too coarse and macroscopic phase separation occurred upon standing at room temperature overnight.

A generally effective strategy to improve emulsion stability is to reduce the average size of the dispersed phase.[56, 62] This effect can be achieved either by addition of a third surface active component or with the aid of external energy input through a sufficient amount of shear by e.g. high speed mixing.[63]. In the former case the excess total surface energy is reduced, thereby reducing the thermodynamic disadvantage deriving from an increase of total interface area associated with the emulsification. On the latter case, energy promotes interface build up. In both cases, only a kinetic stabilization can be achieved. However, this may be sufficient for the purpose of stabilizing emulsions long enough to allow the formation of finely interdispersed blends of two incompatible polymers in the subsequent drying step. The preferable option would be to avoid the introduction of additives that may represent an undesired additional cost and a complication in the assessment of the technological properties and aging stability of the resulting polymer blends. Therefore, experiments were carried out by simply increasing the shear energy during emulsification using an Ultraturrax homogenizer.

In runs C_5T_{60} and $C_{10}T_{60}$ (Table 1) the Gel and PBSA solutions pre-heated at 40°C were combined and homogenised under high shear for 60 sec. Stable emulsions, that is, without visible phase separation occurring overnight, were obtained with both 5% and 10% aqueous gelatin solutions. The short homogenization time and high shear were also effective in preventing gelification of the continuous aqueous phase during the emulsification procedure. Upon standing overnight at room temperature, no macroscopic phase separation was observed. However, optical microscopy observation of the emulsions showed the presence of microbubbles, consistent with the observed volume increase caused by air incorporation during the homogenization.

In order to promote removal of the air bubbles, additional experiments were performed by stirring the as prepared foamy emulsions with a magnetic rod (runs $C_5T_xS_y$). As shown in Figure 2 A-D, after 40 min stirring at 30°C (run $C_5T_{60}S_{60}$) no residual bubbles could be detected by optical microscopy. However, the average size of the dispersed droplets of organic phase after 40 minutes of post treatment was larger than in the as prepared emulsion (Figure 2 C), indicating the occurrence of droplets coalescence. As shown in Figure 3, the size of the organic phase droplets in the $C_5T_{60}S_{60}$ emulsion increase from an average 3.5 μm to 8.7 and 9.0 μm after 40 and 60 minutes of stirring at 30 °C, respectively).

Since the emulsion (kinetic) stability is expected to improve upon reduction of the average size of the dispersed phase, a few experiments were performed to check the influence of the high shear mixing time on the size of dispersed phase. In particular, two experiments ($C_5T_{90}S_{60,}$ $C_5T_{120}S_{60}$) were performed by increasing to 90 and 120 sec, respectively, the homogenization time. In both cases, the size of the dispersed phase right after homogenization was comparable to that of $C_5T_{60}S_{60}$ (Figure 3). However, differently from run $C_5T_{60}S_{60}$, by stirring at room temperature (about 20 ± 2 °C) instead of 30 °C, even after 1 h of post-treatment no significant increase of the size of the dispersed organic phase could be

observed (Figure 3). On the other hand, increasing the homogenization time to 120 sec resulted in a larger incorporation of air bubbles that could not be completely removed even after 1 h of post-treatment (see Figure S3 in supplementary information).

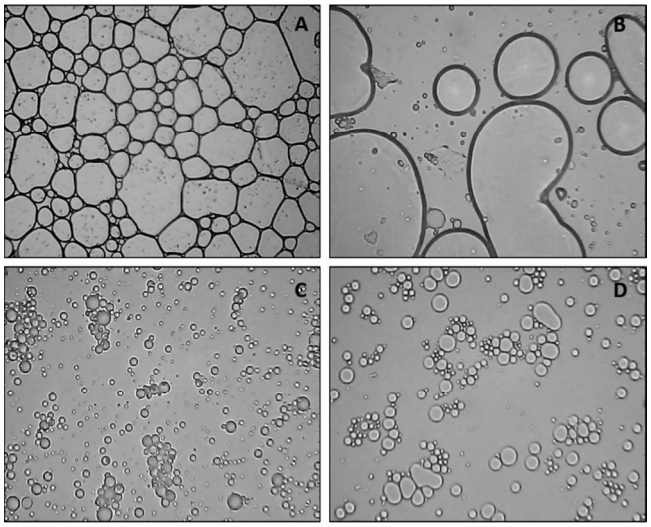

Figure 2: Optical micrographs of emulsion $C_5T_{60}S_{60}$: (A) and (B) as prepared (4x and 40x magnifiction, respectively); (C) after 40 min (40 x) and (D) after 60 min stirring (40x).

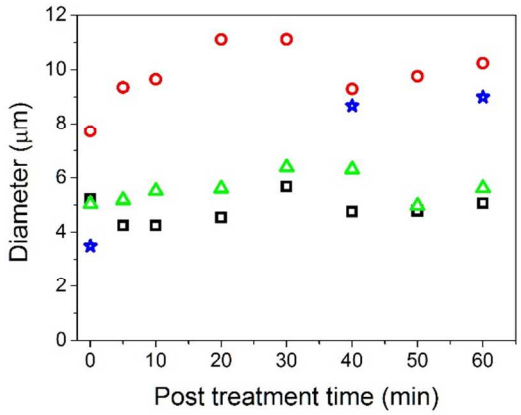

Figure 3: Average diameter of the dispersed organic phase droplets in $C_5T_{60}S_{60}$ (blue stars), $C_5T_{90}S_{60}$ (black squares), $C_5T_{120}S_{60}$ (green triangles), and $C_3T_{120}S_{60}$ (red circles) emulsions at different post treatment times, as obtained by optical microscopy analysis.

The above results indicate that a lower temperature (20 °C instead of 30 °C) during stirring in the post-homogenization process is beneficial for stabilizing the dispersed phase. In fact, the higher viscosity of the continuous aqueous gelatin phase at 20°C provides additional kinetic stability to the emulsion, resulting in inhibition of coalescence. Accordingly, reduction of the gelatin concentration to 3 wt% (run $C_3T_{120}S_{60}$) resulted in an increase of the average size of the dispersed phase along with the reduction of the aqueous phase viscosity (Figure 3).

Repeated preparations based on run $C_5T_{90}S_{60}$ allowed detecting the presence of scattered residual bubbles even after the post-emulsification stirring procedure. To ensure their complete removal, a sonication step (30 minutes in ultrasound bath) was added in the post treatment of run $C_5T_{90}S_{60}U_{30}$, whereas in run $C_{5,10}T_{90}S_{60}U_{30}$ a more concentrated 10 wt% PBSA solution (up from the previous 5 wt%) was used to reduce the overall amount of organic solvent in the emulsion formulation. In both cases, stable emulsions were obtained with no bubbles detected by optical microscopy inspection.

Emulsified blends and blend films

A series of gelatin/PBSA emulsions containing variable amounts of PBSA in the 5-30 wt% (of total polymer content) range were prepared (runs G95P5, G90P10, G80P20 and G70P30, Table 1) following the same emulsification and post-treatment procedure as in run $C_{5,10}T_{90}S_{60}U_{30}$. All compositions gave stable emulsions that could generally be cast into nearly transparent films (Figure 4). Only sample G70P30, with the highest PBSA content (30 wt%), gave a film with a whitish opaque appearance and an upper surface much rougher than the lower surface in contact with the casting substrate.

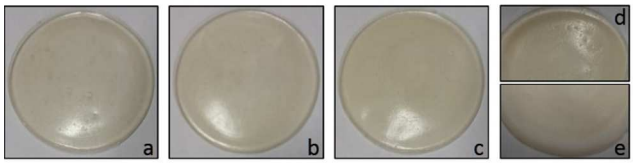

Figure 4: Pictures of sample films of gelatin/PBSA with different compositions: (a) G95P5; (b) G90P10; (c) G80P20; G70P30 upper (d) and lower surface (e).

Inspection of G70P30 film surfaces by scanning electron microscopy showed the presence of large spherical cavities with size in the 100 μm range located at the lower film surface (Figure 5). Micrometer-sized spheroidal heterogeneities line the edges of such cavities (Figure 5d and 6d). On the contrary, the smooth upper film surface had a relatively uniform appearance, with few scattered micrometer-sized spherical heterogeneities (Figure 5f).

A similar morphology was observed in the lower surface of films G90P10 and G80P20 (Figure 5c-d), although with a progressive reduction of the density of the micro-cavities and of the amount of the micro-beads lining their edges along with the reduction of the PBSA fraction in the blend. In agreement with the above observations, in films G95P5 and G100R, containing only 5 % and 0 % PBSA, respectively, such cavities couldn't be detected any more (Figure 5a and 5b).

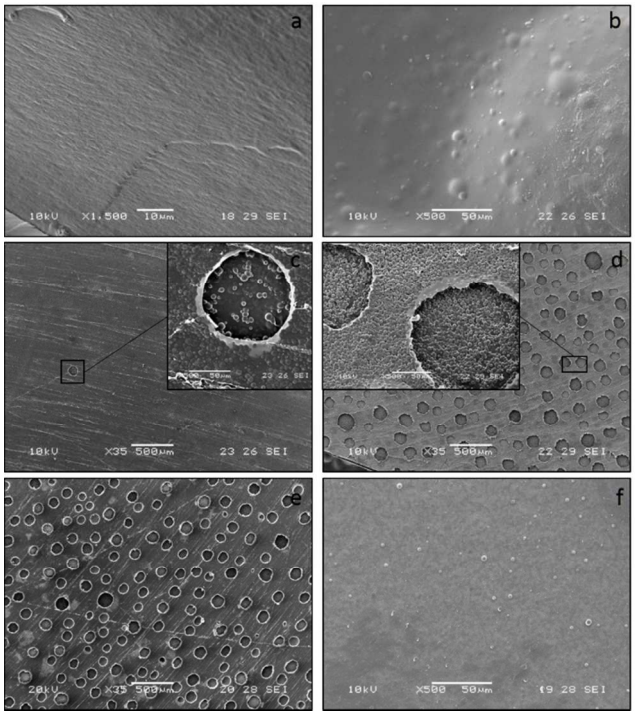

Figure 5: Scanning electron microscopy images of the lower (bottom) and upper (top) surfaces of gelatin/PBSA blend films with different compositions: (a) G100R (top); (b) G95P5 (top); (c) G90P10 (bottom); (d) G80P20 (bottom); (e) G70P30 (bottom); (f) G70P30 (top).

Contrary to such apparent excess concentration of PBSA at the lower side of the films, the ATR-FTIR spectra recorded from the two film surfaces of each sample showed that the concentration of PBSA in the outer layer (roughly 2 μm depth explored by the ATR-FTIR technique) was comparatively higher in the upper than in the lower surface. This is shown by relative intensities of the PBSA carbonyl absorption at 1711 cm^{-1} in the representative ATR spectra of film G70P30. (Figure 6).

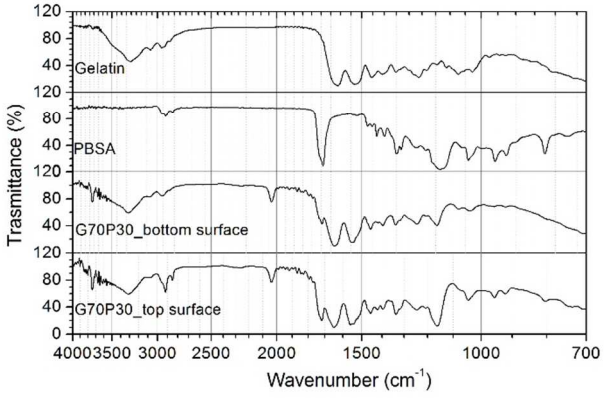

Figure 6: Comparison of the ATR-FTIR spectra of pure gelatin and PBSA films (obtained by compression moulding) with those of the as-cast films G70P30 (lower and upper surface). Spectra are normalized with respect to the maximum intesity carbonyl (ester and amide for PBSA and gelatin, respectvely) absorption between 1600 and 1750 cm^{-1}.

The SEM analysis of the film cross-sections of samples G95P5 (Figure 7a) and G90P10 showed a biphasic morphology consisting of phase-separated spheroidal domains. Their size ranges between 1 and 5 μm and they appear to be homogeneously dispersed throughout the film thickness. On the other hand, samples G80P20 and G70P30 revealed a bilayer morphology (Figure 7b and 7c), with the upper layer similar to that of samples G95P5 and G90P10 and the lower layer presenting large cavities opening up at the lower film surface. Such cavities appear as mainly composed of particles (similar to the dispersed domains in the upper layer) that are either densely dispersed in a continuous matrix or highly aggregated to form a sheathing layer on the inner surface of the cavities (Figure 7d). The concentration of the dispersed domains increases with the increase of the PBSA fraction from 5 to 30 wt%, suggesting that PBSA be the main component of the dispersed phase/particles. The larger thickness of the lower particle-rich layer in sample G70P30 than in G80P20 further supports the ascription of such particles to nearly pure PBSA (see also Supplementary information, Figure S4). Accordingly, neither such layered morphology nor the surface cavities are detectable in the samples with PBSA ≤ 10 wt%.

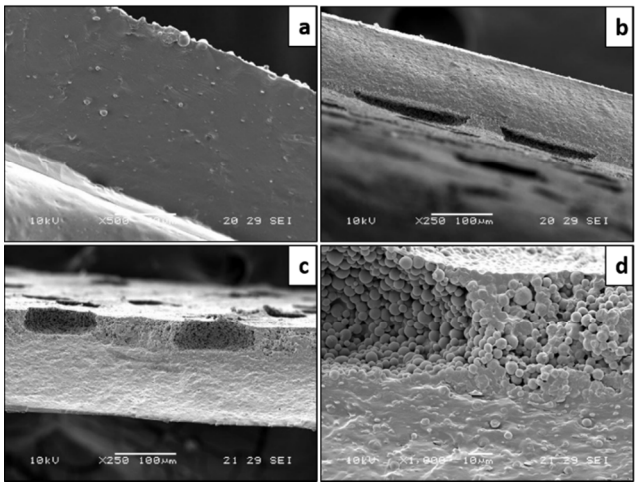

Figure 7: SEM micrographs of the cryofractured sections of gelatin/PBSA cast films: (a) G95P5; (b) G80P20; (c) G70P30; (d) magnification of G70P30 section near the lower surface.

To unequivocally identify the nature of the phase-separated particle-like domains, sample G70P30 was cryofractured and extracted with dichloromethane, a selective solvent for PBSA. The SEM images of the etched cryosection in Figure 8 clearly show that the particle-like domains are effectively and selectively removed by the organic solvent. FTIR analysis confirmed the presence of PBSA as the only component in the extracted fraction (see Supplementary information, Figure S4).

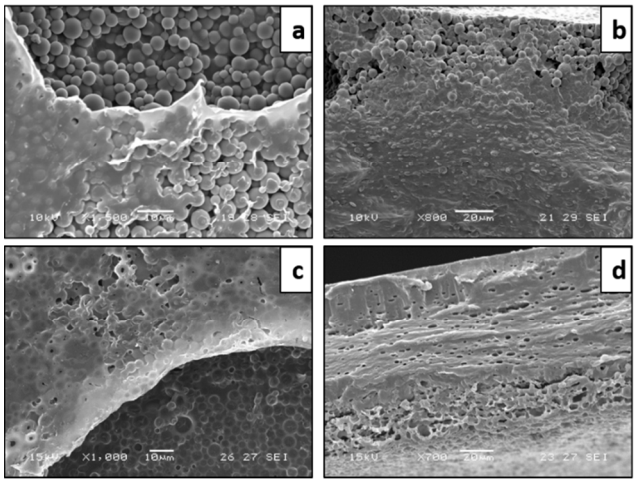

Figure 8: Comparison between SEM micrographs of the the lower surface (a and d) and of cryofractured sections (b and d) of G70P30 cast film before (a and b) and after (c and d) etching with dicloromethane.

Effect of PBSA concentration

The above results show a clear correlation between the fractional amount of PBSA solution in the emulsion and the fractional volume of the cavities. However, they do not allow to clearly identify the driving force for the cavities build-up; in fact, the latter could be promoted either by an increasing fractional amount of organic solvent in the emulsion, or by an increasing weight fraction of PBSA in the polymer blend, or both. In order to clarify this issue two gelatin/PBSA emulsions, both with 90/10 polymer composition, were prepared by feeding the aqueous gelatin with PBSA solution diluted from the 10 % of the previous ones down to 4.4 % ($G90P10_{4.4}$) and 2.6 % ($G90P10_{2.6}$), respectively. The two resulting emulsions had the same CH_2Cl_2/H_2O volume ratios as the emulsions of runs G80P20 and G70P30. The morphology of the films from emulsions $G90P10_{4.4}$ and $G90P10_{2.6}$ turned out to be very similar to that of films G80P20 and G70P30, with large cavities on the lower side and a cross-section in which a bilayer structure was clearly detectable. The average size of the cavities increased from about 250 μm to 1 mm upon dilution of the initial dichloromethane solution of PBSA from 4.4 to 2.6 %, even at constant fraction of PBSA in the dry polymer blend. This clearly indicates that the latter parameter is uninfluential in the generation of cavities.

To further elucidate the role of the organic solvent, two samples were prepared by allowing dichloromethane to evaporate completely from the emulsion before film casting. Film G90P10T was obtained from the same initial emulsion as in run G90P10, but under thermostated condition at 25 °C during the various stages of the preparation to promote nearly quantitative evaporation of the organic solvent before casting. Film G70P30R was obtained from the same initial emulsion as in run G70P30, but in this case, the organic solvent was quickly removed by gently rotating the emulsion under mild vacuum at room temperature before casting. None of the films showed the presence of cavities by SEM

inspection of the lower surface. The morphology of the films in the bulk and on the surfaces was comparable, with a dispersed phase consisting of nearly spherical particles homogeneously distributed in a continuous matrix (Figure 9).

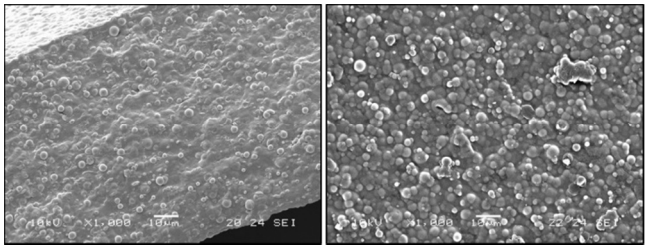

Figure 9: Scanning electron microscopy images of sample G70P30R: cryogenic section (left), surface (right)

The infrared spectra of the upper and lower surfaces were perfectly overlapped, indicating the presence of both gelatin and PBSA in comparable weight ratio within the same film.

Mechanical properties

All gelatin/PBSA blend films were more flexible and less brittle than that of pure gelatin. Accordingly, stress-strain analysis showed a progressive decrease of the Elastic Modulus, E, and of the stress at break, σ_b, along with an increase of the amount of PBSA in the blend (Table 2). The elongation at break, ε_b, showed a less straightforward correlation with the PBSA content. In particular, while ε_b of G90P10 was nearly twice the value of that of pure gelatin, a further increase of the PBSA content in the blend did not result in a corresponding increase of ε_b. Actually, the ε_b value of G70P30 was even lower than that of pure gelatin. Such results are most likely due to the presence, in G70P30, of large cavities acting as nucleation and propagation sites for crack lines, leading to incipient specimen fracture. Accordingly, sample G90P10T, which is characterized by an uniform morphology at a micrometer scale, showed a behaviour comparable to that of pure gelatin, thus suggesting that the mechanical properties are affected mostly by the presence of cavities rather than by the presence and content of PBSA.

Table 2: Tensile properties

Run	E (GPa)	σ_b [1] (MPa)	ε_b [2] (%)
G100	2.1 ± 0.4	60 ± 20	4 ± 2
G90P10	1.9 ± 0.4	60± 10	7 ± 3
G80P20	1.1 ± 0.2	35 ± 9	6 ± 2
G70P30	1.0 ± 0.3	17 ± 4	1.6 ± 0.5
G90P10T	2.4 ± 0.5	40 ± 18	3.7 ± 1.9

[1] Tensile strength; [2] Elongation at break;

Discussion

The results from SEM analysis showed the presence of cavities on the lower surface of the blend films that were cast from emulsions still containing significant amounts of CH_2Cl_2. These cavities are internally coated with a layer of spherical PBSA beads. When the cavities are smaller than 50-100 μm, they appear to favourably affect the mechanical properties of the film by providing additional mechanical flexibility (decrease in the Elastic Modulus and increase in elongation at break) and acting as a toughening agent. This interpretation of the observed mechanical behaviour is confirmed by the differences in the measured tensile properties of G90P10 and G90P10T, with only the former containing a few cavities. On the contrary, when the cavities are larger than 200-300 μm, as in the case of G70P30, they act as stress concentrators,[64] in agreement with the general behaviour typical of fracture mechanics showing a dependency of the stress at break σ_f on the cracks radius a (radius of a spherical defect nucleating crack growth) according to equation (1).

$$\sigma_f \propto \frac{1}{\sqrt{a}} \qquad (1)$$

The film morphology plays thus a crucial role in determining its mechanical properties; in turn, the morphology depends on the preparation conditions such as temperature, mixing procedures, concentration of the emulsified solutions, and residual organic solvent before casting.

The processing temperature and gelatin concentration affects the continuous phase viscosity and thus the kinetic stability of the emulsions. Temperature also affects the evaporation rate of the organic solvent, a parameter already known to be a critical one in determining morphology and ultimate mechanical properties of this kind of heterogeneous films.[56] It is thus important to select a highly volatile one to achieve fast removal from the emulsion and to prevent cavities formation. On the other hand, if a film morphology with asymmetrically distributed cavities is required, for instance to improve film flexibility, a less volatile organic solvent may suit the purpose. The mixing procedure is critical for emulsification but it also promotes unwanted incorporation of air bubbles, which are stabilised by presence of surface active polymers.[65-66] Possibly the most important parameter controlling the film morphology and ultimately its mechanical properties is the residual organic solvent before casting.

In the cartoon of Figure 10 a mechanism for cavities formation is proposed. In the first stage of high shear emulsification a finely dispersed emulsion is obtained (Figure 10A), in which the observed broad size distribution of the organic phase droplets may be ascribed to a Pickering stabilization[66] that involves only a fraction of the droplets where incipient precipitation of PBSA has occurred as a result of

solvent evaporation. The nearly spherical shape of the mostly uncoalesced PBSA particles precipitated within the cavities upon solvent evaporation suggests that a minor amount of gelatin, a mildly amphiphilic polypeptide behaving as a surfactant, be present in the PBSA-rich dichloromethane droplets. The presence of a thick layer of PBSA microspheres even at the upper cavity surface is easily explained by the lower density of PBSA (about 1.25 g/mL) with respect to that of the solvent (about 1.33 g/mL[67]). During film casting, preferential segregation of the less effectively stabilized, largest dichloromethane droplets results in their sedimentation at the bottom of the film as the consequence of the higher density of the organic phase (Figure 10B). The resulting overconcentration of droplets near the lower film interface promotes their coalescence (Figure 10C).

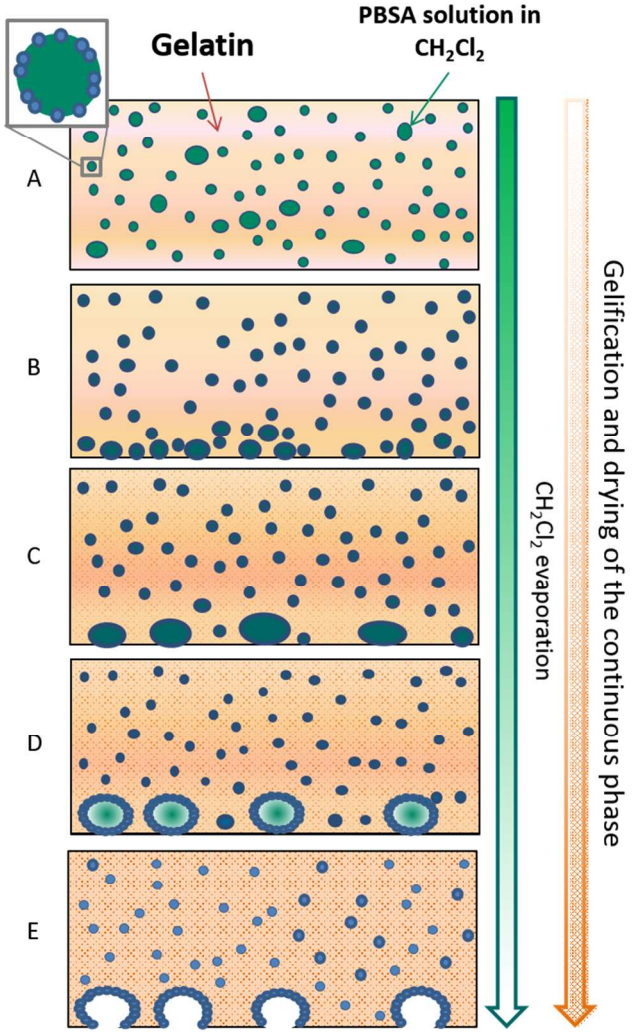

Figure 10: Proposed mechanism of cavity formation in gelatin/PBSA films cast from water/CH_2Cl_2 emulsion: A) oil-in-water emulsion consisting of PBSA/CH_2Cl_2 droplets of variable size dispersed in an aqueous gelatin continuous phase and (possibly) stabilized by PBSA beads precipitated within the organic phase (Pickering emulsion); B) migration of the largest droplets toward the bottom surface of the film under gravity force; C) coalescence of the larger particles densely distributed at the bottom of the gelling film; D) self-segregation of the PBSA beads at the water interface due to further precipitation of PBSA upon evaporation of dichloromethane; C) full gelification of the continuous phase, resulting in stabilization of the cavities generated by the complete evaporation of the organic phase.

Throughout the film drying process the highly volatile dichloromethane, even if not miscible with water, undergoes relatively fast diffusion through the aqueous phase owing to its residual solubility (1.72 wt%[68]) (Figure 10D). The resulting relatively slow evaporation of the organic solvent occurs along with the transition of the aqueous gelatin phase from liquid-like to a strong gel[69], and then a relatively rigid solid upon water evaporation to film dryness. Once gelled, the continuous aqueous phase builds up sufficient mechanical strength to prevent bulk shrinking of the film that may result from collapse of the cavities left behind by the faster evaporation of dichloromethane (Figure 10E). Furthermore, the evaporation of dichloromethane results in precipitation of PBSA from supersaturated organic phase in the form of microspheres, with an amphiphilic character that promotes their migration towards the interface with the aqueous phase. The resulting uniform sheathing of the inner walls of the cavities with PBSA spheres, as shown by SEM analysis, further supports the proposed mechanism of cavity mechanical stabilization. In the proposed mechanism PBSA microspheres act initially as Pickering stabilizers of the oil-in-water emulsion, then as an inner wall for the cavities. Finally, once the droplets start converting into cavities they provide extra mechanical stability to cavities themselves, preventing their collapse under capillary forces.

The ATR-FTIR results showed the presence of a thin outer layer less rich in PBSA at the lower surface of the films cast from emulsions still containing residual dichloromethane. Such a feature supports the hypothesis of the formation of cavities and PBSA beads by a process occurring during the early stage of film drying. In fact, if such preferential segregation was driven by a different affinity of the blend components for the casting PTFE substrate, one would had expected to find a comparatively higher concentration of the less hydrophilic PBSA at the lower film surface.[70] On the other hand, when dichloromethane had been completely removed before casting, the upper and lower film surfaces had the same composition. This suggest a mechanism of PBSA depletion from the lower film surface connected with the presence of large cavities. In fact, according to the mechanism depicted in Figure 10, the growth of cavities is mainly the result of coalescence of smaller droplets that are found at higher concentration at the bottom of the film soon after casting. During this process, the PBSA particles dispersed in the gelatin matrix close to the lower film surface may be incorporated by the coalescing dichloromethane droplets, eventually contributing to the formation of the inner layer of PBSA beads at the cavities walls. This would explain the PBSA depletion observed by ATR, since this technique can only reveal species directly in contact with the IRE crystal.[71]

Conclusion

Heterogeneous films of the hydrophilic gelatin and hydrophobic PBSA were successfully prepared by casting from oil-in-water emulsions obtained by blending aqueous gelatin, pre-heated at 40 °C, with PBSA in dichloromethane under high shear. Several factors contributed to the kinetic stabilization of obtained emulsions; among them, the increasing viscosity of the continuous aqueous phase upon cooling from 40 °C to room temperature during the emulsification and film casting stages, the mild surface activity of gelatin, and possibly a Pickering mechanism resulting from the incipient precipitation of PBSA within the supersaturated droplets of the highly volatile dichloromethane. The most favourable concentration of the aqueous gelatin solution was found at 5 wt%, resulting in a viscosity low enough at 40°C to allow effective mixing under high shear, but also in its adequate increase upon cooling down to room temperature during emulsification and casting. The emulsification conditions optimised for a 80/20 gelatin/PBSA blend were extended to prepare gelatin/PBSA blends with PBSA ranging between 5 and 30 wt%. The morphology of the films prepared by room temperature casting of the resulting emulsions was found to be largely affected by the fractional amount of dichloromethane in the original emulsion. In particular, the organic solvent was observed to promote the formation of large cavities, most of them opening up at the lower side of the films. The formation of such cavities could be prevented by thorough removal the volatile organic solvent before casting, e.g. by mild heating or vacuum pre-treatment of the emulsion. However, such cavities were also found to positively affect the mechanical properties of the films that turned out to be less brittle and more flexible (lower modulus and higher deformability), at least when the largest dimension of the roughly oblate ellipsoidal shape of each cavity did not exceed 100 μm.

The gelatin-PBSA heterogeneous films obtained by simple casting from emulsion may find interesting applications where biodegradable, biocompatible and/or renewable polymeric materials are desired, such as e.g. in agriculture and biomedical applications. Furthermore, the described wet processing approach may be considered as a general method to prepare heterogeneous hydrophilic-hydrophobic films from blends of bio-based and/or biodegradable polymers.

SUPPORTING INFORMATION

ATR spectra of surfaces and SEM micrograph of a cryogenically fractured section of a 80/20 gelatin/PBSA film produced by solvent casting from a TFE solution; glass transition temperature from DSC analysis for gelatin/PBSA blends at different composition prepared from TFE solutions; optical microscopy images of emulsion samples after 5 minutes and 1 h of post-treatment after homogenization;

SEM micrographs of the cryofractured sections of a 90/10 gelatin/PBSA film (G90P10) obtained from a water emulsion; FT-IR spectra of the extracted from G70P30 by washing with dichloromethane

Author Contributions

All authors have given approval to the final version of the manuscript.

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